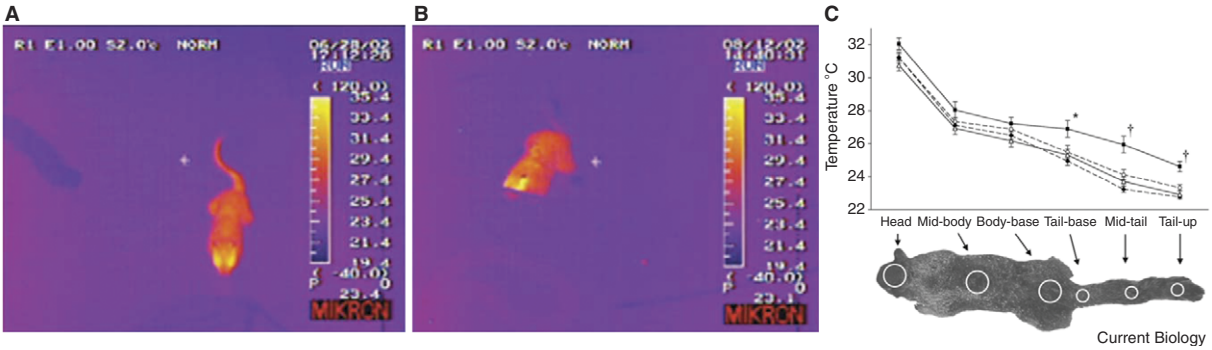


Figure 1. The temperature of ground squirrel tails in response to predator type.

Frames from a thermal imaging camera of a ground squirrel confronted with (A) a rattlesnake, and (B) a gopher snake. Yellows, oranges and reds correspond to warmer temperatures, blues and violets correspond to cooler temperatures. (C) Ground squirrel body temperatures in response to rattlesnakes (squares), gopher snakes (triangles), and two controls: other ground squirrels (diamonds) and baseline (circles). Significant differences in temperatures are found only in the tail region; temperatures are warmest in response to rattlesnakes. (Adapted with permission from [2]; copyright (2007) National Academy of Science, USA.)

production by a sender, how effectively it travels through a medium, and the sensory and cognitive capabilities of the receiver. While studies of animal communication have traditionally emphasized the role of the sender and the medium in signal transmission, until recently less attention has been paid to the role of the receiver [5].

The receiver's sensory capacities serve as filters for the incoming signal. Signals that excite the sensory systems of their intended receivers should be favoured by selection. Numerous examples of signaling within a species show this to be the case. From fish to frogs to fiddler crabs, studies of sexual selection demonstrate that males that produce signals matching females' sensory sensitivity are most successful in obtaining mates (for example, [6–8], reviewed in [9]). Rundus *et al.* [2] have shown that the success of sensory specificity extends to prey–predator communication as well. Prey that communicate to predators in the modality they best perceive can successfully deter attack.

The new study of Rundus *et al.* [2] reminds us that, when studying animal behavior, we must be careful not to confine ourselves to the senses that we ourselves can perceive. In the case of ground squirrels signaling to rattlesnakes, the tail's thermal warning is undetectable to humans and to other ground squirrels alike, but to rattlesnake predators it serves as a potent deterrent. The lesson being, in signal evolution as well as in our study thereof, it pays to be aware of the perceptual world of the receiver.

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# Spindle Microtubules: Getting Attached at Both Ends

**A recent study describes a novel role for the centrosomal protein Cep57 in attaching spindle microtubules to both kinetochores and centrosomes, suggesting similar mechanisms may be used for generating these two distinct linkages in mitosis.**

**Jennifer G. DeLuca**

In a eukaryotic cell, chromosome segregation occurs on the mitotic spindle, a dynamic array of microtubules which requires the function of numerous proteins at centrosomes, kinetochores and along spindle microtubules (Figure 1A). Chromosomes must attach to spindle microtubules via their kinetochores and maintain persistent linkages to these microtubules throughout mitosis. A

recent study [1] has identified the 57 kDa protein Cep57/Translokin as a new component at this interface and demonstrated its requirement for stable kinteochore–microtubule attachment in cycled *Xenopus* egg extract spindles. *Xenopus* Cep57 also functions to tether minus-ends of spindle microtubules to centrosomes, suggesting the linkages at opposite ends of a microtubule may be mechanistically similar.

The linkage that provides stable, end-on attachment between kinetochores and spindle microtubules is likely quite complicated. It must be strong enough to resist the forces produced during chromosome bi-orientation, but also flexible enough to allow for constant addition and loss of tubulin subunits at attached microtubule plus-ends [2]. Extensive work in many systems has shown that the Ndc80 complex, a heterotetramer composed of protein subunits Ndc80/Hec1, Nuf2, Spc24 and Spc25, is essential for the robust kinetochore–microtubule attachments required for chromosome bi-orientation [3–7]. The purified recombinant Ndc80 complex directly binds microtubules *in vitro*, albeit with low affinity [8,9]. The binding affinity is increased by addition of KNL1 and Mis12 complexes, known associates of Ndc80. It is still unclear, though, how the Ndc80 complex and its partners coalesce to form functional binding sites for the plus-ends of dynamic microtubules.

In budding yeast, the Dam1 complex has surfaced as an excellent candidate for providing robust, yet flexible linkages from kinetochores to microtubules. Subunits of this ten-protein complex are required for chromosome congression and for attaching microtubule plus-ends to kinetochores in budding yeast cells (reviewed in [10]). *In vitro*, the ~210 kDa complex forms dimers which, upon addition of microtubules, form rings and spirals around the microtubule lattice [11,12]. These rings remain bound to polymerizing and depolymerizing microtubules, even in the presence of substantial loads on microtubule ends [13], lending support to the idea that Dam1-complex rings may be kinetochore–microtubule attachment factors in cells. No homologs of the 39 kDa Dam1 protein have been identified outside of yeasts, nor have rings encircling microtubules been observed by electron microscopy in cells [14], which has inhibited the mitosis field from fully embracing Dam1-complex rings as a universal mechanism for kinetochore–microtubule attachment. Emanuele and Stukenberg [1] suggest in their new paper that Cep57 may be a Dam1 homolog, as it shares some degree of sequence homology with Dam1, and its functions in *Xenopus* egg extract spindles resemble those proposed for Dam1 in budding yeast mitosis [1,10].

Emanuele and Stukenberg [1] showed that *Xenopus* Cep57, the human homolog of which was originally identified in a proteomics screen for centrosomal proteins [15], localizes to kinetochores, centrosomes and to microtubules in cycled *Xenopus* egg extract spindles [1]. Antibody-mediated depletion of Cep57 resulted in loss of end-on kinetochore–microtubule attachment in these spindles. *In vitro* kinetochore–microtubule binding assays using isolated chromosomes and purified tubulin confirmed this finding, as addition of Cep57 antibody reduced the number of chromosome-bound microtubules. Blocking both Ndc80 and Cep57 function did not produce an additive effect, leading the authors to conclude that Cep57 and Ndc80 are in the same kinetochore–microtubule attachment pathway. Cep57 is downstream of Ndc80, as Ndc80 was retained at kinetochores after Cep57-depletion, whereas Cep57 failed to localize to Ndc80-depleted kinetochores. Thus, Ndc80 is present at Cep57-depleted kinetochores, but is unable to generate stable kinetochore–microtubule attachments.

How does Cep57 contribute to the formation of these attachments? At least two possibilities can be considered: in the first, Cep57 functions as the budding yeast Dam1 homolog and, in concert with its binding partners, forms ring-like structures around microtubules, which may mediate attachment to kinetochores in cells (Figure 1B). Cep57, like Dam1, localizes to kinetochores, centrosomes, and spindle microtubules [1,10]. Cycled *Xenopus* egg extract spindles depleted of Cep57 not only exhibited kinetochore attachment defects, but also structural defects, as the spindles incorporated ~40% less tubulin than control spindles. This is noteworthy, as Dam1 plays a role in spindle assembly and structure in budding yeast [16]. Dam1 directly binds microtubules (reviewed in [10]), and while Emanuele and Stukenberg [1] did not demonstrate direct microtubule binding, Cep57 co-sedimented with polymerized microtubules from *Xenopus* egg extracts. The authors suggest that, although sequence homology between the two proteins is limited, Cep57 may functionally serve as the higher eukaryotic Dam1 homolog. This assessment would be consistent with the finding that Cep57 and the Ndc80 complex are in the same pathway for generating stable kinetochore–microtubule attachments, as the Ndc80 complex has been suggested to bind the microtubule lattice through Dam1 in budding yeast [17]. *Xenopus* Cep57 is part of a large and uncharacterized complex, so it will be important to identify the complex components and determine if they represent the homologs of Dam1-complex members. A clear next step will be to test if the Dam1-complex microtubule-binding mechanism is conserved across eukaryotes by expressing the entire Cep57 complex *in vitro* and assaying its ability to form load-bearing rings around microtubules.

Cep57 may alternatively contribute to formation of kinetochore–microtubule attachments by serving as a cross-linker or scaffold at the attachment site (Figure 1C). In this scenario, Cep57 connects either microtubules to

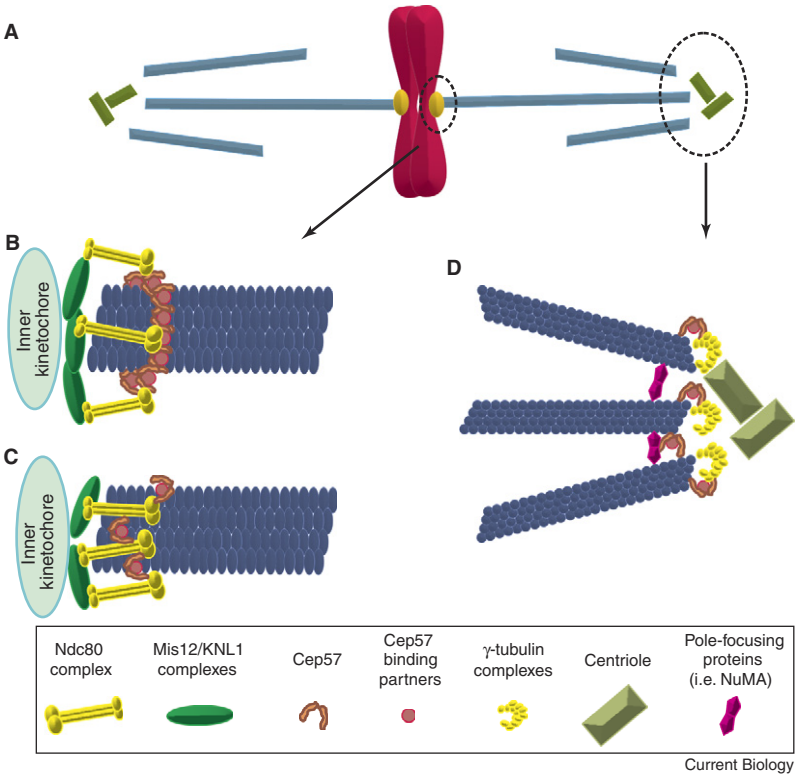


Figure 1. Models for Cep57-mediated linkages at both ends of spindle microtubules.

(A) In mitosis, microtubules must dynamically link to both centrosomes and kinetochores. (B) Cep57 may function as the higher eukaryotic Dam1 homolog and form rings around the plus-ends of kinetochore microtubules in concert with its complex members. If analogous to budding yeast, the Ndc80 complex links to the microtubule through its connections with the Cep57 complex. (C) Alternatively, Cep57 may function to either concentrate or correctly position kinetochore–microtubule attachment factors such as the Ndc80 complex by cross-linking these proteins to each other or to the microtubule lattice. (D) Cep57 also plays a role in dynamically linking the minus-ends of microtubules to centrosomes. Cep57 may bind both microtubule minus-ends and γ-tubulin-containing centrosome complexes. This model would allow for dynamic exchange of tubulin at the minus-ends of microtubules. By providing a tethering function between microtubule ends and either centrosomal proteins (D) or kinetochore proteins (B,C), Cep57 may utilize similar mechanisms for anchorage at both ends of spindle microtubules.

kinteochore-bound attachment factors or kinetochore-bound attachment factors to themselves, which may produce higher affinity binding sites for microtubules. Antibodies to Cep57 immunoprecipitated several kinetochore proteins, including Ndc80 and known Ndc80-interactors Zwint and Mis12 [1]. Cep57 may serve to either concentrate kinetochore–microtubule attachment factors to levels required for stable microtubule binding or position attachment factors in the correct orientation to produce stable linkages. In support of this, Ndc80 complex purified from *Caenorhabditis elegans* was found to bind microtubules with low affinity, which is increased by raising the concentration of Ndc80 complex in the reaction or addition of purified KNL1 and Mis12 complexes [8]. The Cep57 complex may play a similar role in cells by increasing the binding affinity of the Ndc80 complex for microtubules. Emanuele and Stukenberg [1] point out that Cep57 has two coiled-coil domains separated by a linker region, suggesting it has the geometry to provide a flexible linkage between proteins. Cep57 may link the Ndc80 complex to microtubules or link Ndc80 complexes together, thereby concentrating it around the microtubule lattice. It may be preliminary, however, to predict a structural role for Cep57 without knowing the identities or structural predictions of its binding partners.

On the other end of the microtubule, minus-ends must remain focused during mitosis via tethers to other microtubule minus-ends or to components of the centrosome to maintain spindle integrity [18]. Similar to plus-ends, this linkage must be flexible to allow for dynamic exchange of tubulin subunits, particularly to allow for microtubule disassembly, which is required for poleward flux [19]. Emanuele and Stukenberg [1] suggest that Cep57 participates in this linkage, as its depletion from extracts produces defects in centrosome-mediated aster formation. Live-cell imaging demonstrated that aster formation was initially normal, but microtubules failed to remain tethered to the centrosome and floated away from the aster. This effect was specific to centrosome-induced asters, as spindles formed via DNA-coated-bead addition or by the addition of constitutively active Ran were not affected by Cep57 depletion. The Cep57-mediated minus-end linkage may involve γ-tubulin, as it is immunoprecipitated by Cep57 antibodies. The authors suggest that Cep57 may cross-link the minus-ends of microtubules to γ-tubulin-containing complexes within the centrosome to provide a flexible linkage at the minus-ends in a manner similar to that proposed for the plus-ends (Figure 1D). In the future it will be important to test the role of Cep57 in spindle assembly in other cellular systems: since *Xenopus* extract spindles can rely on multiple mechanisms for assembly [18], a more severe Cep57-depletion phenotype may be observed at spindle poles in cells which rely heavily on centrosome-mediated spindle assembly.

Emanuele and Stukenberg's [1] interesting study on Cep57 has placed a new player at the attachment interfaces on both ends of mitotic microtubules. It will be fascinating to see what the future holds for this protein and its associated partners, specifically at the plus-ends of

kinetochore microtubules. Are rings conserved at higher eukaryotic kinetochores which use many microtubules to make an attachment, or does the single microtubule at the budding yeast kinetochore require a unique structure to maintain the delicate balance of a firm, yet flexible grip?

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# Respiratory Biology: They Would Be Giants

**A recent study has shown that respiratory structures are disproportionately oversized in larger insects, and that oxygen supply to leg muscles may be physically constrained in the largest modern insects. High oxygen concentrations during the Carboniferous may have alleviated these physical constraints allowing the evolution of gigantic arthropods.**

**John R.B. Lighton**

Oh, those giant late Carboniferous arthropods, how they captivate the imagination of writers and the curiosity of scientists. Meter long millipedes! And those wingspans: 70 centimeter dragonflies, 45 cm mayflies! With the discovery that atmospheric $O_2$ levels spiked as high as 35% during the peak of insect gigantism, the mystery was partly solved [1,2]. Evidently, high $O_2$ concentrations may have overcome the insects' presumed respiratory limitations. However, hard data to suggest that modern insects are limited in size by present-day $O_2$ concentrations close to 21% were lacking. Yes, modern dragonflies fly better in synthetic Carboniferous air than in the modern atmosphere [3], but that doesn't directly address the gigantism question. In many other insects, high $O_2$ concentrations show no effect. A good way to address the question, as it turns out, is to look in depth at the scaling between gas transport mechanisms and body size.

When most people think about gas transport, they think lungs and bloodstream. In mammals, lung volume is a constant fraction of body volume, irrespective of size [4]. But not all animals have lungs, and thereby hangs an exoskeleton. Take insects, for example. Lungs, heart, and bloodstream have they none; at least not in vertebrate terms. Their circulatory systems lack the respiratory pigments on which $O_2$ and $CO_2$ hitch-hike in vertebrates. Instead, they have air-tubes called tracheae that transport respiratory gases directly to and from their tissues, neatly bypassing the retail supply chain. Insects don't breathe in the sense that vertebrates such as mammals do. So, how do the volumes of their tracheal systems scale with the volumes of their bodies? Like many questions in basic insect physiology, this question, amazingly, has been examined only very recently.

From the work of Jon Harrison and collaborators [5], we know that grasshoppers invest disproportionately more in their tracheal volumes as they grow from infants to adults, but these results are complicated by the fact that adult hoppers deploy expensive accessories such as wings and the muscles required to power them. Now, Alexander Kaiser and